Resistance Mutations to NS3 Protease Inhibitors and Quasispecies Diversity in HCV Chronically Infected Patients Treated with Pegylated Interferon and Ribavirin Using Next Generation Sequencing.

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INTRODUCTION: Hepatitis C treatment with pegylated interferon and ribavirin (PEG-IFN/RBV) results in sustained virological response in only 50% of the treated patients. New compounds are being developed and most advances were obtained with protease inhibitors (PIs). Resistance mutations are observed in most cases. Since before the beginning of PIs therapy it is important to verify the presence of resistance mutations in both majority sequences and in quasispecies to assess possible emergent resitants. This work aims to detect, in a dynamic approach, quasispecies of HCV and PIs resistance mutations from HCV chronically infected patients treated with PEG-IFN/RBV. MATERIAL AND METHODS: 68 patients with chronic hepatitis C, genotype 1, were selected from Hepatology Service of the Federal University Hospital Clementino Fraga Filho, Rio de Janeiro. Blood samples were collected at different time points (pre-treatment, 48 hours, 7 days, 30 days and 3 months), viral RNA was extracted and RT-nested PCR from partial NS3 protease gene were done. PCR-amplified products were directed sequenced to identify the majority sequences. Three-dimensional molecular models from NS3 proteases were constructed. To analyze the quasispecies, PCR-amplified products were sequenced by next generation sequencing (NGS) using Ion Torrent technology. RESULTS AND DISCUSSION: The analyses of majority sequences from three patients showed the presence of the following resistance mutations: patient 1, classified as non-responder, T54S; patient 2, relapser, V36L and V55A; and patient 3, sustained virological responder, T54S. HCV quasispecies are being analyzed by bioinformatics tools and preliminary results showed high diversity among patients, but conserved pattern of virus variability in samples of the same patient before and after PEG-IFN/RBV treatment. CONCLUSION: Assessment of NS3 protease gene sequences before the PI therapy allow the knowledge of majority and minority sequences with resistance mutations that can influence in anti-HCV treatment efficacy.

Key words: hepatitis C virus, quasispecies, next generation sequencing, NS3 protease, bioinformatics

Supported by: CNPq, INCT-INPeTAm/CNPq/MCT, CAPES, FAPERJ/PPSUS/MS